

## APPARENT CHANGES IN THE ABUNDANCE AND DISTRIBUTION OF *ANOPHELES* SPECIES ON GRENADA ISLAND<sup>1</sup>

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**ABSTRACT.** Results of a recent survey of the 3 species of *Anopheles* mosquitoes, *Anopheles* (*Nyssorhynchus*) *aquasalis*, *Anopheles* (*Nyssorhynchus*) *argyritarsis*, and *Anopheles* (*Anopheles*) *pseudopunctipennis*, on the island of Grenada are contrasted with the only other published survey of these species on Grenada, which was published in 1938. Results suggest that a significant change in the abundance and distribution of each species has occurred over this period. Physical characteristics of the aquatic habitats for each species are described and compared.

### INTRODUCTION

Grenada Island, located in the Eastern Caribbean, is the most southerly of the Windward islands, situated 12°N and 61°W. The island is 19 km wide, 34 km long, and lies 160 km north of the Venezuela coast. Grenada is a volcanic island and Mount St. Catherine is the highest peak at 840 m above sea level. The mountainous and thickly wooded island has a large number of streams and rivers. Intensive agriculture, the major activity on the island, has changed the environment in some areas from forests to numerous fields of cocoa, nutmeg, bananas, various spices, fruits, and vegetables. The tropical climate of Grenada is characterized by a distinct dry season from January through May and a wet season from June through December.

*Anopheles* (*Nyssorhynchus*) *aquasalis* Curry, *Anopheles* (*Nyssorhynchus*) *argyritarsis* Robineau-Desvoidy, and *Anopheles* (*Anopheles*) *pseudopunctipennis* Theobald are the 3 species reported from the few anopheline surveys conducted in Grenada. *Anopheles argyritarsis* was the only *Anopheles* species reported by Macdonald (1916). Howard et al. (1917) mentioned that *Anopheles* (*Nyssorhynchus*) *tarsimaculata* Goeldi (= *An. aquasalis*), *An. argyritarsis*, and *An. pseudopunctipennis* were collected in Grenada. Root and Andrews (1938) in a comprehensive wet season survey collected the same 3 species with the following comparative abundance: *An. aquasalis*, 18.7%; *An. argyritarsis*, 30.0%; and *An. pseudopunctipennis*, 51.3%. Two species, *An. aqua-*

*salis* and *An. pseudopunctipennis*, have been documented as important vectors of human malaria parasites in many countries of Central and South America (Simmons 1941). Throughout its range *An. pseudopunctipennis* is generally the dominant vector in mountainous areas during the dry season (Shannon et al. 1927, Aitken 1945, Rodriguez and Loyola 1989). In 1991 we initiated a study on the population genetics of this species from the southern United States to northern Argentina, including the Caribbean region.

*Anopheles pseudopunctipennis* was first described by Theobald (1901) from Grenada Island (Lesser Antilles). The original description was not adequate for accurate identification of the species. This resulted in confusion and misidentifications of the species in other parts of its geographical range in North, Central and South America. Three names currently in synonymy, 5 subspecies and one variety have been applied to this species in South America (Knight and Stone 1977). *Anopheles* (*Anopheles*) *franciscanus* McCracken was also confused with this species for many years. Aitken (1945) stated: "No description of the egg, larval, and pupal stages, or of the male terminalia of *An. pseudopunctipennis* have been made from the type-locality" (Grenada). Objectives of our study in Grenada were to collect topotypic specimens of *An. pseudopunctipennis*, to redescribe and fully illustrate the species in all life stages, and to study its present abundance and distribution on this island.

### METHOD OF SURVEY

The larvae of *An. pseudopunctipennis* are normally found in pools of drying streams and river beds, thus our survey was conducted in April 1992 during the dry season. We collected along streams and rivers inland and throughout the coastal area, except for the southeastern coast where no suitable habitats were found (see map, Fig. 1). We sampled 100 aquatic habitats at elevations from 3 to 300 m. Specimens from 40 collections of immature stages (larvae and pupae)

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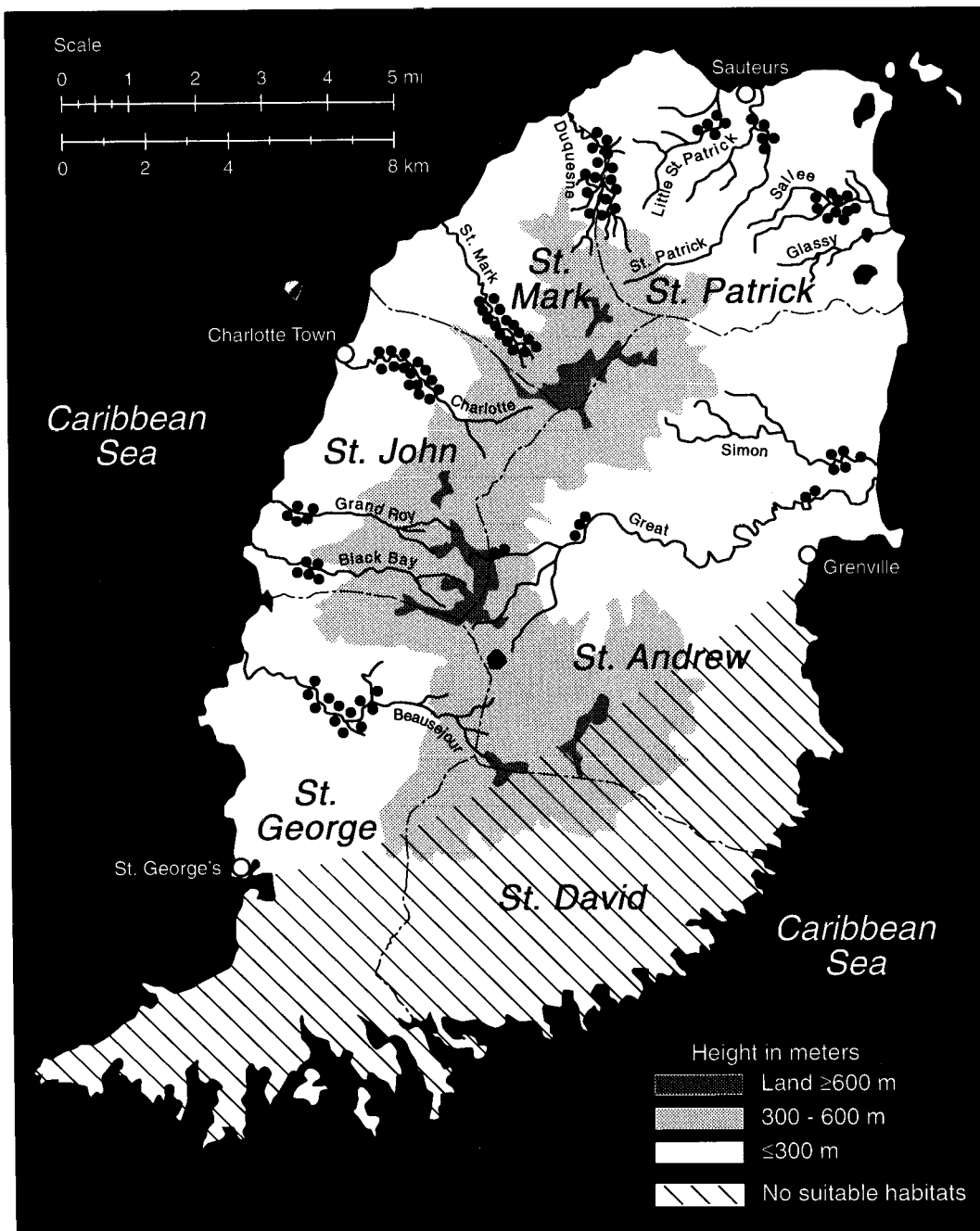


Fig. 1. Collection sites on Grenada, April 1992. The 100 sampling sites are indicated by dark circles.

and 3 collections of adults attracted to humans were retained for molecular, biochemical, and taxonomic studies. The immatures were reared individually to the adult stage, larval and/or pupal exuviae preserved, and each specimen re-

corded. Each adult mosquito was identified in the field; a group of adults from each collection was frozen for genetic studies and some were pointed on pins for taxonomic vouchers. In addition to the 40 habitats mentioned above, other

aquatic habitats were positive for *An. argyritarsis* larvae. Following preliminary identifications of larvae from the latter habitats the specimens were discarded.

## RESULTS

**Type of habitat:** The type and distribution of larval habitats are heavily influenced by the topography of this mountainous island, and particularly by heavy rains at higher elevations. In most areas the hills rise abruptly from the ocean. River networks cover the entire island. During the dry season the large number of streams and rivers are reduced mainly to side pools, minimal water flow, and isolated pools, affording an abundance of oviposition sites. There are also a few small mangrove swamps and 3 lakes or ponds in old craters that are permanently filled with water. Of the latter habitats, one was negative for *Anopheles* and 2 were not accessible.

Most immatures of *An. aquasalis* and *An. argyritarsis* were collected in stream pools with an abundance of floating dead leaves. *Anopheles argyritarsis* was also frequently associated with mats of green filamentous *Spirogyra*-type algae. *Anopheles pseudopunctipennis* was found along stream margins and was closely associated with 2 types of green algae. The most common was a green filamentous alga belonging to the genus *Cladophora*. This alga occurred in large, thick mats in the Saltee River. The second was a green "lettucelike" alga belonging to the genus *Enteromorpha*. This alga is known as an indicator of brackish water.

**Permanence of oviposition sites:** Only 20% of the oviposition sites found in April 1992 were permanent bodies of water. All *An. pseudopunctipennis* specimens were collected from permanent bodies of water, compared to 33% and 15% of *An. aquasalis* and *An. argyritarsis* specimens, respectively. This finding represents a difference from the results of Root and Andrews (1938), who found *An. aquasalis* more often associated with permanent oviposition sites (92%) than *An. pseudopunctipennis* (66%) or *An. argyritarsis* (56%).

**Elevation:** *Anopheles pseudopunctipennis* was found only near sea level, and *An. argyritarsis* was collected from near sea level up to 300 m. Most *An. aquasalis* populations occurred near sea level with only one population being collected at 180 m. These observations differ from those of Root and Andrews (1938) who found *An. aquasalis* at elevations not exceeding 30 m and *An. pseudopunctipennis* being collected up to 120 m above sea level.

**pH and conductivity:** pH and conductivity measurements were taken on site using a portable

pH/conductivity meter. The variations in pH found for the oviposition sites of the 3 *Anopheles* were not significantly different, with range limits of 5.90–7.98. Readings of water conductivity for *An. argyritarsis* and *An. aquasalis* larval habitats varied from 400 to 4,000  $\mu$ S. Water conductivities for *An. pseudopunctipennis* habitats were relatively constant at 4,000–4,500  $\mu$ S. The Saltee River, where *An. pseudopunctipennis* larvae were collected, was fed by mineral springs rich in various salts.

**Water current:** *Anopheles argyritarsis* and *An. aquasalis* larvae were most common in stagnant water habitats, whereas *An. pseudopunctipennis* larvae were found only in slow moving waters with thick mats of green algae. This finding seems to be in contradiction with Root and Andrews's (1938) statement: "All three species of *Anopheles* were more frequently found in still water than in a current, and *An. tarsimaculatus* (*An. aquasalis*) and *An. pseudopunctipennis* were almost restricted to water without current." However, the thick mats of green algae in which *An. pseudopunctipennis* larvae and pupae were found in our survey offered strong protection against the slow water current.

**Abundance of the 3 anopheline species:** Table 1 shows a breakdown of all 100 oviposition sites sampled. Of the 100 sites, 72 were positive for *An. argyritarsis*, 13 were positive for *An. aquasalis*, and 11 were positive for *An. pseudopunctipennis* larvae. Four sites had both *An. argyritarsis* and *An. aquasalis* larvae, one site had both *An. argyritarsis* and *An. pseudopunctipennis* larvae, and 8 sites had all 3 species.

**Distribution of the 3 *Anopheles* species (Fig. 1):** *Anopheles argyritarsis* was the most ubiquitous species in Grenada. This species was found from near sea level up to an elevation of 300 m, in temporary or permanent oviposition sites, in water with various pH and conductivities, associated with green filamentous algae or not, in shaded and unshaded habitats, in stream pools, stream margins, and rock pools of almost all streams and rivers surveyed (Table 1). *Anopheles aquasalis* was mainly found in the northern part of the island, especially in St. Patrick Parish. *Anopheles pseudopunctipennis* populations were found in a very small and specific area of St. Patrick Parish, in the northeastern part of the island. The populations of *An. pseudopunctipennis* were found in 2 different rivers located in the same area. One population represented by one specimen was found in the Glassy River and the 10 other populations were from the Saltee River where most of our *An. pseudopunctipennis* specimens were collected. The proportions of the 3 species present in the Saltee River were 88% *An. pseudopunctipennis*, 10% *An. aquasalis*, and 2% *An. ar-*

Table 1. Presence and abundance of the 3 *Anopheles* species on Grenada (frequency of species in parentheses).

Location	Number of collections	Location of collecting sites	<i>Anopheles pseudopunctipennis</i>	<i>Anopheles argyritarsis</i>	<i>Anopheles aquasalis</i>
Beausejour River	12	SW	—	10 (99%)	2 (1%)
Black Bay River	5	W	—	—	—
Grand Roy River	5	W	—	—	—
Charlotte River	15	W	—	13 (100%)	—
St. Marks River	15	NW	—	13 (100%)	—
Duquesne River	15	NW	—	13 (100%)	—
Little St. Patrick River	5	N	—	4 (47.5%)	1 (52.5%)
St. Patrick River	5	N	—	—	1 (100%)
Sallee River	10	NE	10 (88%)	8 (2%)	8 (10%)
Glassy River	1	NE	1 (7%)	1 (93%)	—
Great River	7	E	—	6 (99%)	1 (1%)
Simon River	5	E	—	4 (100%)	—
Total	100		11%	72%	13%

*gyritarsis* (Table 1). Larval habitats of all 3 species in the Sallee River were located along the margins of the river, which had a slow water current and a high salt content. Larvae and pupae were concentrated on top and inside of thick mats of green filamentous algae. The mats served as both shelter and food for the larvae (Hoffmann and Samano 1938).

## DISCUSSION

Our observations indicate that the abundance and distribution of the 3 species of *Anopheles*, and especially larval habitats of *An. pseudopunctipennis* and *An. argyritarsis*, were very different from those reported by Root and Andrews (1938). The comparative frequency of encountering each of the 3 species went from 51.3% to 11.0% for *An. pseudopunctipennis*, from 30.0% to 72.0% for *An. argyritarsis*, and from 18.7% to 13.0% for *An. aquasalis*. In our survey the most abundant and ubiquitous species was *An. argyritarsis*, which was found in association with *An. aquasalis* in the littoral zone. *Anopheles argyritarsis* was the only species collected above an altitude of 180 m. In the case of *An. pseudopunctipennis*, Root and Andrews (1938) reported the species on the northwestern part of the island, whereas we collected the species only in a small northeastern area of the island. We made a large number of collections along the Charlotte and St. Marks rivers, in the regions of Gouyave and Victoria where Root and Andrews (1938) found abundant *An. pseudopunctipennis* mosquitoes. Although we found many suitable *An. pseudopunctipennis* larval habitats, our collections were

uniformly negative for this species in both regions. Admittedly there were seasonal differences between our survey and the survey of Root and Andrews (1938). However these differences do not seem to account for the complete absence of *An. pseudopunctipennis* larvae in many seemingly optimal larval habitats.

This study shows important variations from the data of Root and Andrews (1938) that reflect changes in the ecology of the *Anopheles* mosquitoes in Grenada. During the intervening 55 years, the widely distributed populations of *An. pseudopunctipennis* appear to have become confined to a single small area of Grenada. Isozyme analyses (Manguin et al. 1993<sup>5</sup>) show less genetic variability of *An. pseudopunctipennis* populations in Grenada (percent polymorphic loci = 3.1%) compared to the continental populations from Central and South America (average percent polymorphic loci =  $19.1 \pm 4.3\%$ ). The reduced genetic variability of *An. pseudopunctipennis* populations on Grenada Island may reflect a bottleneck effect due to reproductive isolation. The lack of genetic variability might have reduced the adaptability of *An. pseudopunctipennis* populations to environmental changes, resulting in the species' diminished presence on the island. In contrast, environmental changes on the island seem to favor the proliferation of *An. argyritarsis*. This species has become numerically dom-

<sup>5</sup> Manguin, S., E. L. Peyton and R. Fernandez Loayza. 1993. Population genetics of *Anopheles pseudopunctipennis*, vector of malaria in Central and South America. Unpublished manuscript presented at 59th Annual Meeting, American Mosquito Control Association, Ft. Myers, FL.

inant and widely distributed. Earle (1936) stated: "*An. argyritarsis* has never become abundant in Grenada which probably renders it unimportant as a malaria vector." Root and Andrews (1938) concluded that *An. aquasalis* was probably the major vector of malaria in Grenada because of its corresponding distribution with malaria cases. Although Grenada is currently a malaria-free country (PAHO 1992), *An. pseudopunctipennis* and *An. aquasalis* are known vectors. Although no recent data incriminate *An. argyritarsis* as a vector, its ubiquity and abundance on the island could possibly catapult it into a vector role if malaria were reintroduced to Grenada. Regardless, the potential for autochthonous malaria infections in Grenada is assured by the continued presence of the other 2 proven vector species, viz., *An. aquasalis* and *An. pseudopunctipennis*.

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